66 660.51 S993

Synthetic Organic Chemicals

PUBLISHED BY THE
EASTMAN KODAK COMPANY, ROCHESTER, N. Y.

VOL. X APRIL, 1937 No. 2

The Role of Amino Acids in Growth

S. S. FIERKE

Research Laboratories—Eastman Kodak Company

UNTIL 1931, only four of the twenty-one amino acids known to be present in proteins had been proved to be indispensable dietary components. These were lysine, tryptophan, cystine, and histidine. Previously, progress in protein chemistry had been fairly slow until the advent of the Kossel and Kutscher procedure for the isolation of the diamino acids, and Fischer's ester method for the separation of the monoamino acids.

Following this, it was soon recognized that proteins of different sources vary enormously in regard to the proportions of their constituent amino acids. Thus, gliadin of wheat proved to be deficient in lysine; zein of corn was shown to be practically devoid of lysine and tryptophan; and gelatin was found to be lacking in tryptophan, tyrosine, cystine, valine, iso-leucine, and hydroxyglutamic acid.

With the recognition of the biochemical importance of the individual amino acids, it was discovered that protein metabolism was a much more complex phenomenon than had been supposed.

Among the early investigations regarding the importance to nutrition of the individual amino acids, those of Osborne and Mendel are of particular value. By observing the growth of young rats fed upon diets containing zein as the sole protein, they succeeded in demonstrating the indispensable nature of both tryptophan and lysine. They noted that the animals receiving zein rations not only failed to grow but quickly lost weight; that the addition of tryptophan to the food resulted in maintenance of weight but no growth, while the inclusion of both tryptophan and lysine was followed by rapid growth. Similarly, when gliadin of wheat was used as the sole protein of the diet, it was found that growth did not occur until lysine was incorporated in the diet. It was not necessary to supply the missing amino acid in the free state. The supplementation of a zein diet with some other protein containing adequate quantities of lysine and tryptophan gave the same results. In their absence, nutrition fails and eventually death results.

In a like fashion, the same investigators showed that cystine also is essential. When 18 per cent of casein was incorporated in an otherwise adequate ration, young rats receiving such a food mixture grew at a normal rate. When the proportion of casein was progressively diminished, however, the cystine present in the material became the limiting factor. At a 9 per cent level, casein was found to be incapable of inducing normal growth, but the addition of cystine rendered the diet adequate. Recently, Jackson and Block discovered that methionine may replace a large part of the cystine.

In 1916, Ackroyd and Hopkins ob-

served that when arginine and histidine are removed from acid-hydrolyzed casein, the resulting material is inadequate for maintenance or growth. The authors state that if either arginine or histidine is included in the ration, no loss in weight occurs, and growth may be resumed. From these results they concluded that the two amino acids are interchangeable in metabolism, but that at least one must be present in the diet. Rose and Cox subsequently confirmed these findings as to the essential nature of histidine.

Methods of Testing

At this stage it appeared that if further information were to be secured regarding the relation of the amino acids to maintenance and growth, either more adequate methods would have to be devised for the quantitative removal of single components of the protein, or new methods formulated to prove the essential or nonessential nature of the amino acids. The three methods in general use for studying the relation of amino acids to maintenance and growth were: (1) To feed a protein devoid of, or deficient in, one of these components; (2) to feed a hydrolyzed protein from which one or more of them had been removed as completely as possible; (3) to feed a mixture of purified amino acids.

The possibilities of determining the nutritive importance of amino acids by the first two methods are quite limited. Of the twenty known protein components, only four, namely, lysine, tryptophan, cystine, and histidine, had been shown by these methods to be essential.

Most of the amino acids used for feeding experiments are derived from natural sources. The lysine, histidine, and arginine are prepared from blood meal or blood corpuscle paste by electrical transport after previous acid hydrolysis. Tyrosine and tryptophan are secured from casein by enzymatic hydrolysis. Cystine is prepared from hair by acid hydrolysis. Proline and hydroxyproline are derived from gelatin by acid hydrolysis. The others are more conveniently prepared synthetically because of difficulties in their separation and purification from natural sources.

At first the feeding of purified amino acids seemed to be of little value in studying the nutritive importance of the individual components because no one had succeeded in obtaining growth on such mixtures. However, Ellis and Rose found that if a combination of nineteen amino acids was supplemented by 5 per cent of casein, gelatin, or gliadin, the animals lost weight for several days and then slowly gained. This was interpreted as evidence of the existence in protein of an unrecognized essential dietary component. Windus, Catherwood, and Rose have shown that this substance is present in the monoamino-monocarboxylic acid fraction of hydrolyzed protein. If concentrates of this material were incorporated in a diet otherwise adequate except for the absence of a single amino acid, one would expect to create a deficiency, provided that amino acid was indispensable.

Phenylalanine and Tyrosine

Womack and Rose fed to young rats mixtures of highly purified amino acids, the diets being supplemented with concentrates of the unknown growth essential discovered by Rose and co-workers, and found that phenylalanine is essential for proper growth of the animal and that tyrosine is dispensable. The replacement of phenylalanine by tyrosine in the diet was followed by rapid loss in body weight, and if phenylalanine was added to the diet in the absence of tyrosine, rapid gains in weight ensued.

For some time evidence has been accumulating that one or more of the leucines may be an indispensable component of the diet. For example, Abderhalden reported that iso-leucine and nor-

AMINO ACIDS

Indispensable	*Dispensable**
Lysine	Glycine
Valine	Alanine
Tryptophan	Serine
Cystine or Methionine	nor-Leucine
Histidine	Aspartic Acid
Phenylalanine	Glutamic Acid
Leucine	Hydroxyglutamic Acid
iso-Leucine	Arginine
α-Amino-β-hydroxy-n-butyric Acid	Citrulline
	Proline
	Hydroxyproline
	Tyrosine

**Research to date makes these appear to be dispensable.*

leucine may be dispensed with, provided leucine is present. Womack and Rose investigated the nutritive values of these amino acids and found that their absence from the ration leads to complete nutritive failure, similar to the result when only one is present in the diet. If all three are present, excellent growth ensues, but leucine and iso-leucine induce similar increases in weight, proving that the latter two are essential components of the diet, while nor-leucine probably is dispensable.

The most important research in some years was reported recently when McCoy, Meyer, and Rose announced the isolation and crystallization of a new essential amino acid, which they identified as one of the four optically active α-amino-β-hydroxybutyric acids. Rose and co-workers found that it is present in casein, fibrin, serum albumin, and serum globulin, but that hemoglobin and free globin prepared from the latter are extremely poor sources of the compound. The pure amino acid has been prepared principally from casein and fibrin by acid hydrolysis.

The chemical nature of this compound has been demonstrated by reduction of the material to d-α-amino-n-butyric acid. Carter has furnished confirmatory evidence of its general nature by the successful synthesis of a mixture of the four optically isomeric α-amino-β-hydroxybutyric acids. The pair of mirror image products synthesized from crotonic acid exerts extremely little, if any, growth stimulation. After epimerization of this pair, the resulting material manifests about one-fourth to one-sixth of the activity of the natural amino acid.

Rose and co-workers have shown that with an otherwise adequate diet, approximately 0.6 per cent of α-amino-β-hydroxybutyric acid is the minimum amount necessary to induce maximum growth. They also were the first to demonstrate successfully that animals are able to grow upon diets including synthetic mixtures of highly purified amino acids in place of proteins.

The amino acids which have been discussed, together with valine, whose value recently has been reported by Rose and co-workers, are known to be necessary for growth in animals. Other essential amino acids may be discovered when the present methods are extended and improved. The table on this page gives the tentative classification of the known amino acids with respect to their nutritive importance as outlined by Rose in the *Harvey Lectures, 1934-1935.*

Melting Point Apparatus

THE usual melting point apparatus involving the use of liquid baths of oil, fused salts, sulfuric acid, etc., has several disadvantages, such as smoking, spattering, and burning. To overcome these objections, Berl and Kuhlmann developed and described in BER. *60*, 811 (1927) a copper block for determining melting points. A modification of this block which has proved very satisfactory in the Eastman Synthetic Organic Laboratories is shown as Figure 1.

This apparatus consists essentially of a copper cylinder with vertical holes for thermometers and for the usual capillary tubes, and horizontal holes for illumination and observation. Illumination of the sample is obtained by means of two Pyrex rods bent in the manner sketched in Figure 2, Top, so that they will transmit the light to the enclosed portion of the capillary tube. The illuminated area is magnified by means of a lens which aids considerably in observing minor changes that may take place, such as shrinkage, decomposition, and so forth.

FIGURE 1—*Melting point apparatus showing thermometer and capillary tube in place.*

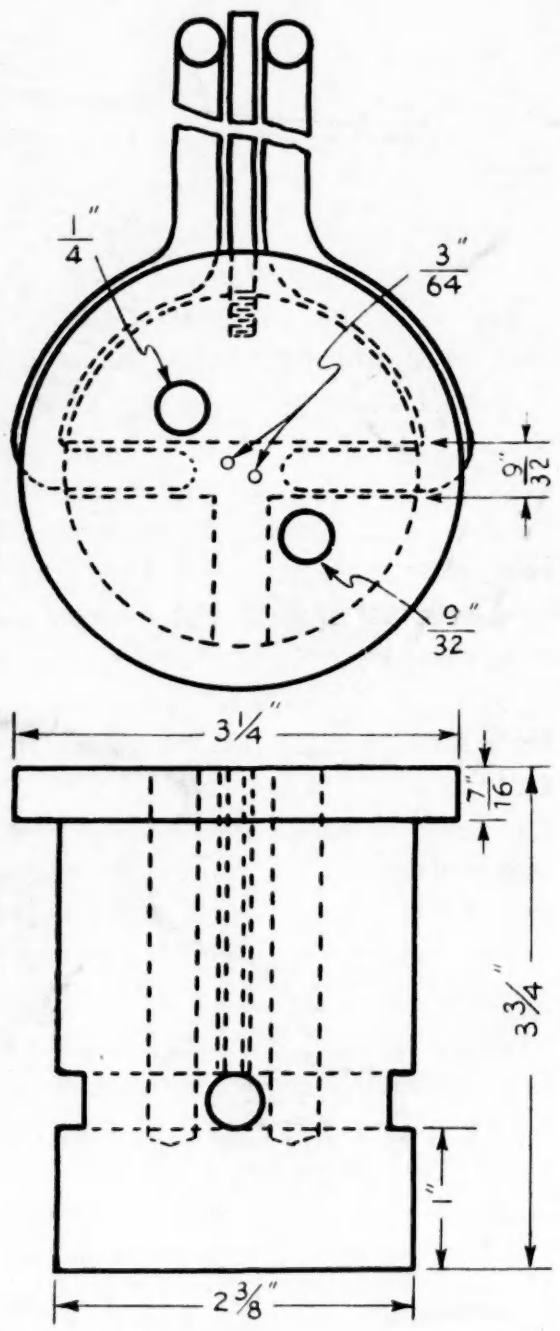

FIGURE 2—*Diagrams of block.* UPPER—*top view;* LOWER—*front view.*